DETECTION AND QUANTIFICATION OF AMPHETAMINE TYPE STIMULANTS AND METHADONE IN HAIR USING LIQUID CHROMATROGRAPHY TRIPLE QUADRUPOLE (LC QQQ) MASS SPECTROMETRY

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By

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
AP	Amphetamine
AP/MA	Amphetamine to methamphetamine ratio
BIONADI	National Narcotics Information System
COC	Cocaine
R^2	Correlation coefficients
CV	Coefficient of variation
CZE	Capillary zone electrophoresis
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EMIT	Enzyme multiple immunoassay technique
FID	Flame ionization
FPIA	fluorescence polarization immunoassay
GC/MS	Gas Chromatography mass Spectrometry
GC/MS/MS	Gas Chromatography Tandem Spectrometry
GC–NCI–MS	Gas chromatography negative chemical ionization mass spectrometry
GC–PCI–MS	Gas chromatography positive chemical ionization mass spectrometry
HCL	Hydrochloric acid
HFBA/HFPOH	N-methylbistrifluoroacetamide/N-methyl-tert-butylsilyltrifluoroacetamide
HILIC	Hydrophilic interaction liquid chromatography
KET	Ketamine
KOH	Potassium hydroxide
LC/MS	Liquid Chromatography mass Spectrometry
LC/MS/MS	Liquid Chromatography Tandem Spectrometry
LLE	Liquid-liquid extraction
LLOQ	Lower limit of quantification
LOD	Limit of detection
LOQ	Limit of quantification
m/z	Mass-to-charge ratio
MA	Methamphetamine
MBDB	N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine
MDA	3,4 methylenedioxyamphetamine
MDE	3,4-methylenedioxyethylamphetamine
MDMA	3,4 methylenedioxymethamphetamine
MMT	Methadone maintenance therapy
MTD	Methadone

6-MAM	6-monoacetylmorphine
NaOH	Sodium hydroxide
NP-FID	Nitrogen phosphor flame ionization detector
NP-LC	Normal phase liquid chromatography
PFPA/PFPOH	Pentafluoropropionic anhydride/pentafluoropropanol
$[M+H]^+$	Protonated molecule
RIA	Radioimmunoassay
RP-LC	Reverse phase liquid chromatography
RSD	Relative standard deviation
SAMHSA	Substance Abuse and Mental Health Service Administration
SPE	Solid phase extraction
SPEM	Solid phase microextraction
SPME	Headspace solid-phase microextraction
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THC	Tetrahydrocannabinol
TLC	Thin layer chromatography
UHQ	Ultra-high quality water

LIST OF SYMBOLS

µg/mL	Microgram per milliliter
µL	Microliter
mg	Milligram
mL	Milliliter
ng/mL	Nanogram per milliliter
ng/mg	Nanogram per milligram
pg/mg	Picogram per milligram

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LIST OF PUBLICATIONS

Poster presentation

SAAD, K., ABDULMANAF, N. and LATIFF, A.A. (2011). Detection and Quantification of Amphetamines and Methadone in hair using Tandem Mass Spectrometry. SoHT annual meeting, Joint symposium with IATDM-CT, Joint symposium with TIAFT, Chamonix 2011 Conference, 21-26 March 2011,Chamonix Mont Blanc, France, P53.

SAAD, K., ABDULMANAF, N. and LATIFF, A.A. (2011). Detection and Quantification of Amphetamines Type stimulants and Methadone in hair using Liquid Chromatography Triple Quadrupole Mass Spectrometry. Advancing Science in the Fight Against Doping symposium, 25-26 May 2011, Doha, Qatar, P2.

PENGESANAN DAN KUANTIFIKASI PERANSANG JENIS AMFETAMINA DAN METADON DI DALAM RAMBUT MENGGUNAKAN KROMATOGRAFI CECAIR SPEKTROMETRI JISIM TRIPLE QUADRUPOLE (LC QQQ)

ABSTRAK

Lebih sedekad yang lalu, analisis rambut dalam penyalah gunaan dadah telah menjadi satu alternatif yang penting berbanding ujan dadah di dalam urin. Analisis rambut dicadangkan sebagai satu petunjuk yang lebih sesuai dalam ujian pengambilan dadah secara kronik kerana dadah terdapat dalam bentuk bebas. Rambut memberi tempoh pengesanan yang lebih panjang dan menjadikannya sukar untuk dimanipulasi. Selain itu, rambut juga mudah untuk dikumpul dan stabil pada suhu bilik dalam jangka masa yang panjang. Analisis rambut semakin meluas digunakan untuk pemeriksaan saringan pekerja, bidang sains forensik, kawalan antidoping dan diagnostik klinikal. Oleh kerana itu, kajian ini adalah bertujuan untuk membangunkan satu kaedah yang sensitif dan khusus untuk mengesan dan menentu-ukur perangsang jenis amfetamina dan metadon dalam rambut dan urin, menentukan korelasi penemuan analisis antara urin dan rambut dan juga korelasi antara penggunaan metadon dan perangsang jenis amfetamina pada penagih. Pembangunan dan validasi kaedah telah dijalankan dengan menggunakan kaedah pengekstrakan cecair diikuti dengan Kromatografi Cecair Spektrometri Jisim Sejajar (LC/MS/MS). Sampel-sampel diekstrak menggunakan kaedah pengekstrakan cecair diikuti hidrolisis menggunakan NaOH dengan kehadiran “deuterated internal standards” (d_3-metadon, d_3-metamfetamina dan d_5-amfetamina). Satu sistem LC/MS/MS yang berdasarkan kepada Kromatografi Cecair Spektrometri Jisim Sejajar bersama Pengionan Elektrosemburan telah digunakan. Kaedah MS/MS telah

dioptimuman dengan melakukan pemantauan pelbagai tindak balas. Bagi tujuan pengesahan, sebanyak tiga ion diagnostik telah dipantau dan ion yang tertinggi dipilih untuk tujuan kuantifikasi dan ion kedua dan ketiga pula digunakan untuk tujuan pengesahan. Pengesahan kaedah telah dilakukan mengikut beberapa garis panduan daripada piawaian antarabangsa ISO/IEC 17025, WADA ISL dan Nota Teknikal NATA. Kaedah pengesahan telah menunjukkan peratus pemulihan adalah lebih daripada 80%. Kelinearan diterbitkan pada julat kepekatan 5 – 100 pg/mg rambut dan regresi untuk semua analit adalah melebihi 0.997. Had pengesanan ialah 1 pg/mg untuk semua sebatian kecuali pada rambut yang dimasukkan metildiamfetamina (MDA) dan amfetamina iaitu sebanyak 5 pg/mg. Kejituan intra-hari dan inter-hari adalah kurang daripada 10%. Dalam kajian ini, 39 sampel rambut dan urin daripada penagih yang telah melalui proses pemulihan telah dianalisis. Kajian telah menunjukkan kepekatan metamfetamina, amfetamina dan metadon masing-masing ialah 0.015 - 109.95 ng/mg, 0.005 - 8.45 ng/mg dan 0.008 - 45.22 ng/mg. Di samping itu, kajian menunjukkan bahawa tiada korelasi antara tahap dadah dalam analisis rambut dan urin, seterusnya menunjukkan analisis rambut boleh digunakan sebagai penunjuk biologikal yang lebih baik kepada penggunaan dadah tunggal, kerap ataupun secara kronik berbanding dengan analisis dalam urin. Kesimpulannya, kaedah yang dibangunkan dengan menggunakan LC/MS/MS merupakan satu kaedah yang sangat sensitif dan khusus untuk pengesanan dan kuantifikasi amfetamina dan metadon.

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ABSTRACT

Over the last decade, analysis of hair for drugs of abuse has emerged as one important alternative to urine drug test. Hair analysis has been proposed as a more suitable marker of past chronic drug intake as drugs are present in the free form; hair provides a longer detection window making it difficult to cheat ; it is easy to collect and the sample is stable at room temperature for long period of time . Hair analysis is increasingly used in pre-employment and employment screening, in the forensic sciences, doping control and clinical diagnostics. Therefore, the aim of this study is to develop a sensitive and specific method for the detection and quantification of amphetamine type stimulants (ATS) and methadone in hair and urine, establish a correlation between the findings of urine and hair analysis in addicts and establish a correlation between the use of methadone and ATS in addicts. A method was developed and validated using liquid–liquid extraction followed by Liquid Chromatography Tandem Mass Spectrometry. The samples were extracted using liquid-liquid extraction following hydrolysis with NaOH in the presence of deuterated internal standards (d_3-methadone,d_5-methamphetamine, and d_5-amphetamine). A bench top LC/MS/MS system based on a Liquid Chromatography Triple Quadrupole (LC QQQ) Mass Spectrometry with Electrospray ionization (ESI) was used. The optimization of MS/MS method was developed using the multiple reaction monitoring (MRM). For the purpose of confirmation, three diagnostic

ions were monitored, the most abundant of which was chosen for the purpose of quantification and the second two were used for confirmation purposes. Method validation was performed according to several guidelines that include the international standards ISO/IEC 17025, WADA ISL and NATA Technical Note. The validated method showed that the recoveries were greater than 80%. Linearity was established over the concentration range of 5 – 100 pg/mg hair and the regression for the individual analytes were all greater than 0.997. The limit of detection (LOD) obtained was 1pg/mg for all compounds except for 3,4 methylenedioxyamphetamine (MDA) and amphetamine was 5 pg/mg in spiked hair. The intra and inter day precision was generally less than 10% in spiked hair. In this study, thirty nine hair and urine samples from rehabilitated drug addicts were analyzed. The concentrations of methamphetamine, amphetamine and methadone were 0.015 - 109.95 ng/mg, 0.005 - 8.45 ng/mg and 0.008 - 45.22 ng/mg, respectively. The study showed that there was no correlation between drug levels in urine and hair analysis, indicating hair analysis can be used as a better biological indicator to prove single use, occasional and chronic drug abuse compared to urine. As a conclusion**,** the developed method using LC/MS/MS was found to be highly sensitive and specific for detection and quantification of amphetamines and methadone.

CHAPTER 1
INTRODUCTION

1.1 Background and Rationale

Drug abuse has been recognized as a widespread problem around the world. Patterns of this problem are constantly changing. They differ significantly on the basis of the region, from country to country, and to the geographical areas within the same country (Wills, 2005). Since the 1980s, drug abuse has been of a great concern in Malaysia and has been identified as one of the major problems that threaten the national security (Manaf, 2002).

The severity of drug abuse can be determined from the number of new and recurring cases of drug addicts as indicated by the record from the National Narcotics Information System (BIONADI) of the National Narcotics Agency of the Ministry of Home Affairs, Malaysia. This agency manages the record of all incidences of drug addiction cases since 1970 (Manaf, 2002). Statistics of drug addicts detected throughout the country for the period from January to December 2010 showed that a total of 23,642 addicts have been detected; this includes 17,238 new addicts and 6404 relapsed addicts representing about 50.24% increase of over the same period the previous year. Also, the largest increase was the amphetamine type stimulants amounting 5,001.19% increase compared to the previous year (AADK, 2010). Due to the significant increase in drug abuse, the state intensified its efforts in increasing awareness and creating a society that is free of drugs through adapting the Anti-Drug Education Program at schools,

workplace, universities, etc. The Ministry of Education has organized these programs at schools through the tools of mass media, seminars, pamphlets, and exhibitions, and through promoting healthy outdoor activities and others. However, the Ministry of Health is responsible for identifying drug addicts and ensuring the implementation of the detoxification programs. All institutions of the state, both governmental and non-governmental organizations, should contribute to various activities to increase a public awareness about drugs.

The use and abuse of illegal drugs affects all modern communities and therefore assessment of drug exposure is an important concern that needs to be accomplished. So, the detection of drugs of abuse in biological samples is one the primary functions of a clinical or forensic toxicology laboratory. Urine testing is most frequently used for routine testing for drugs of abuse, but many alternative specimens are establishing their place as suitable specimens for drug testing (Khadehjian, 2005). The alternative biological matrices include hair, sweat and saliva and have now reached an adequate standard of scientific credibility to be considered in federally regulated workplace drug testing. The research described in this thesis focuses on the hair analysis for drugs of abuse.

Hair analysis is a developing technology that has become both an alternative and complementary approach to drug abuse detection. Hair analysis provides some advantages over urine analysis. In comparison to urine analysis, the main advantages of hair analysis include (a) it can be used as a marker of past chronic drug intake by providing the retrospective and past periods of drug use that are directly related to the

hair length ;(b) long window detection period of abuse ranging from days to years making it difficult to cheat compared to urine or blood (hours to 2-5 days for most drugs); (c) the sample collection is non-invasive, it is easy to be performed under conditions that prohibit adulteration and substitution (Boumba *et al.*, 2006; Freye & Levy, 2009). In recent years, there have been remarkable advances in sensitive and specific analytical techniques for determination of drugs in hair samples mainly by Gas Chromatography mass Spectrometry (GC/MS) and Liquid Chromatography mass Spectrometry (LC/MS). The recent technology of Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) with sensitivity and specificity allows the detection and quantification of many different drugs in biological matrices and often requires less sample preparation. According to the review by Maurer LC/MS/MS has the potential to become the 'gold standard' for forensic and clinical analysis (Maurer, 2005).

Amphetamine Type stimulants (ATS) has been chosen because it represents a great public health issue. Recently, the consumption of these drugs has rapidly increased among the youth population as recreational drugs (Cirimele, 2006). Methadone has been included in this study because its treatment often leads to a decrease in the level of primary drug use and reflects the use of stimulants among opiates' users (Gossop *et al.*, 2000; Peters & Reid, 1998)

1.2 Research Objectives

Hair has become the third most popular biological matrix that is used for drug analysis after blood and urine. For drugs of abuse, urine and hair are widely accepted as forensic evidence because of the non invasive nature in sample collection. Besides, hair has a wide diagnostic window of detection at very low levels of drugs. Moreover, the ability to establish both chronological profile and severity of drug consumption is possible. For hair analysis, a sensitive method is required to ensure the detection at very low concentration of drugs. The ability to detect drug at very low concentration compared to urine analysis to eliminate false negative. Therefore, this thesis focus on the optimization of a single extraction method and faster separation at low detection limit with good resolution of analytes. To achieve these goals, the present study is focused on the following:

1. Development and optimization of hair analysis using HILIC column.
2. Development of a sensitive and specific method for the detection and quantification of amphetamine type stimulants and methadone in hair using Liquid Chromatography Electrospray Ionization (ESI) Tandem Mass Spectrometry.
3. Establishment of a correlation between drugs concentrations in urine and hair in rehabilitated addicts.

The developed methods will be validated and applied for the detection and quantification of amphetamine type stimulants and methadone in hair.

1.3 Thesis Layout

The main body of this dissertation consists of a general introduction and background, literature reviews, material and methods, results and discussion, conclusions as well as recommendations for future study.

CHAPTER ONE is general introduction on the background of drug of abuse, testing for drug of abuse and the measurable objectives of this study are briefly discussed.

CHAPTER TWO is general literature review which is divided into three major parts: (section 1) the hair analysis for drugs of abuse, (section 2) Drug of abuse particularly Amphetamine Type Stimulant (ATS) and Methadone, and (section 3) the analytical tools for hair analysis.

CHAPTER THREE lists down all the applied materials and methods. The method developments involve development and optimization of instrument and extraction method included urine and hair analysis.

CHAPTER FOUR presents the results and discussions arising from the study.

CHAPTER FIVE is the final chapter which consists of overall conclusions on the whole study and recommendations for the future study on hair analysis for drugs of abuse.

CHAPTER 2
LITERURE REVIEWS

This chapter is divided into three sections. Section 2.1 will provide an overview of the physiology of hair, mechanisms of drug incorporation into hair, and application of hair analysis. Section 2.2 will review the drugs of abuse mainly amphetamine type stimulants and methadone, providing an overview of the principles and kinetics of absorption, distribution, metabolism and excretion. Finally, section 2.3 will review analytical techniques that are used for hair analysis.

2.1 Hair Analysis

Hair analysis for drug of abuse has emerged as both an alternative and complementary approach to urine analysis of drug (Boumba *et al.*, 2006; Cooper *et al.*, 2011; Kintz, 2008; Pragst & Balikova, 2006).

In the 1960s and 1970s, in order to determine the effects of heavy metals, such as arsenic, lead, or mercury, hair analysis was employed. As hair could keep substances for a prolonged time period, researchers gave a proposition that hair was a suitable specimen to blood and urine for evaluating environmental hazards (Boumba *et al.*, 2006; Freye & Levy, 2009). As such, the analysis of overall or segmented hairs might facilitate objective data on the degree of an individual's exposure to metals and its capability in causing harm. Due to inadequate knowledge on the sensitivity of analytical methods

employed for these compounds, the use of hair matrix for organic substances, especially pharmaceuticals and drugs of abuse, was examined several years later. In 1979, Baumgartner and colleagues published the first report using radioimmunoassay (RIA) to detect morphine in the hair of heroin abusers (Baumgartner *et al.*, 1979). Since then, a number of drugs including amphetamines, cocaine, cannabinoids and phencyclidine have been reported to be incorporated in hair. In addition, fetal hair has been used to detect maternal drug abuse during pregnancy. Technically, in comparison to testing many other matrices, testing of hair for drugs is less tedious. More so, in spite of the initial sample preparation, the application of analytical methods and instrumental approaches in several cases is quite similar (Kintz, 2008). The Society of Hair Testing (SoHT) founded in 1995. The goals of SoHT are (a) promotion of research in hair testing technologies in forensic, clinical and occupational sciences, (b) development of international proficiency tests, (c) organization of meetings and workshops and (d) encouragement to scientific cooperation and exchanges among members (Jurado & Sachs, 2003; SoHT, 2004; Pragst & Balikova, 2006). The guidelines of SoHT provide recommended best practice guidelines whether laboratories are planning or currently offering drugs testing in hair. These guidelines include reference to recommended sample collection and storage procedures, through sample preparation, pre-treatment, analysis, and the use of cut-offs (Cooper *et al.*, 2011; SoHT, 1997).

2.1.1 Hair Physiology

2.1.1.1 Structure of Human Hair

Hair is a filamentous biomaterial which evolves from the hair follicle found on the dermis; it is a product of differentiated organs in the skin of mammals. Chemical composition of hair is 65 to 95% protein, 1 to 9% lipids, 0.1 to 5% pigments (melanin) and minute amounts of trace elements, polysaccharides and water (Harkey, 1993; Robbins, 2002).

Every hair shaft is composed of three unique types of cells: an outer cuticle, which encloses a central cortex which may further consist of a central medulla. The cuticle consists of overlapping scale cells that help guide the hair shaft in the follicle and defend the interior fibers. The cortex is composed of spindle-shaped cortical cells and in the core of the cortex there may be condensed cells forming the medulla, which might be continuous or diversified with air spaces. The type of pigment and alignment gives hair its color while pigment granules are also found in cortical cells. For more illustration on the structure of hair follicle, consider Figure 2.1 below.

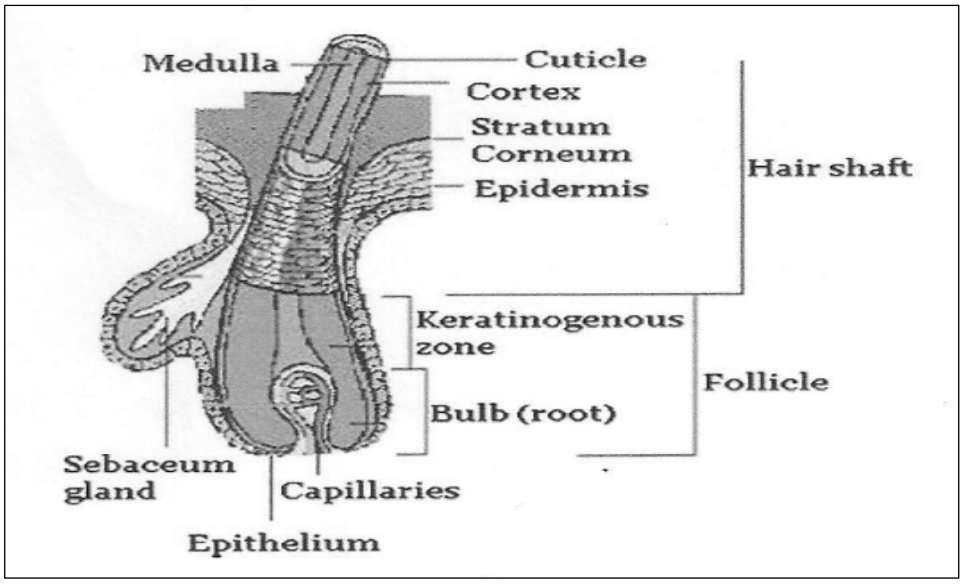

Figure 2.1 Simplified diagram of hair structure (Boumba *et al.*, 2006)

2.1.1.2 The Growth of Human Hair

The growth of human hair relies on several factors such as diet, health, inherited traits, hormone balance, age, physical condition, climate, chemical effects, sex, and the presence of any disease. Switching between the periods of growth (anagen phase) and the periods of quiescence (catagen and telogen phases), the hair shaft begins in a follicle closely connected with glands (sebaceous and apocrine) and grows in cycles (Harkey, 1993; Kronstrand & Scott, 2006).

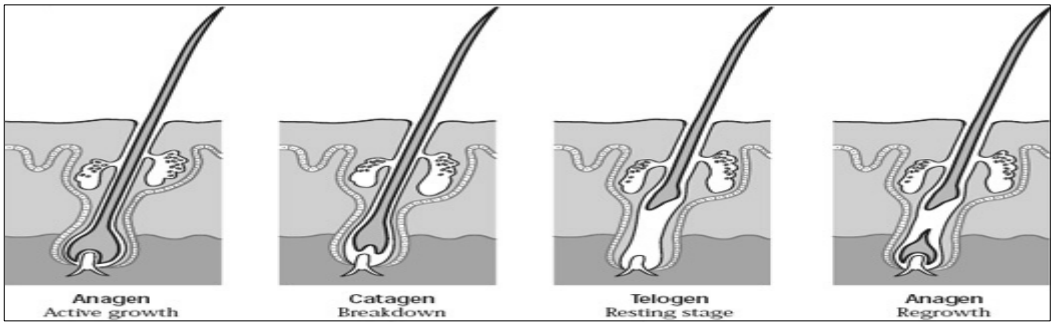

Figure 2.2 The hair growth cycle (One, 2010)

Anagen is defined as the growth period when the follicle mature and hair develop. Generally, the period of anagen phase varies between 7 to 94 weeks but, may also eventually last for many years, depending on the anatomical region (Harkey, 1993). In the case of head hair, it can grow for a period of 4 to 8 years (< 12 months for non-head hair) at a rate proportional to 0.22 to 0.52 mm/day or 0.6 to 1.42 cm/month (Castanet & Ortonne, 1997).

Anagen is followed by Catagen, which is the phase of regression. Cell division stops during this phase, and the changes leads to the base of the hair shaft becoming fully keratinized forming dry, white node characteristic of a ‘club’ hair. It takes 2 weeks for the formation of the follicle bulb, following which it will start to begin degenerate (Harkey, 1993).

Catagen is followed by telogen phase, which is the phase of resting or quiescent period. The growth of the hair shaft totally stops during this phase and remains in the upper portion of the follicular canal whereby it can be easily dislodged by pulling. The duration of this phase increases gradually with age and depends on the body area where

hair is found. In terms of the scalp hair, the resting phase is about 10 weeks which is comparably short and is about 2-6 years for the general body surface. Another growth cycle starts after the telogen phase (Harkey, 1993).

2.1.1.3 Pigmentation

In mammals, melanin is formed in specialized cells called melanocytes, which covers distinct cytoplasmic organelles known as melanosomes. Four stages are involved in Pigment formation (follicular melanogenesis) in the melanosomes (Robbins, 2002). In the first stage, tyrosinase and protein constitute the basic structural unit, in the next stage an inner membranous structure is formed wherein the melanin is biosynthesized and accumulated. At the final stage, the melanosome changes into a uniformly dense melanin particle. The pigmented hair shaft is formed when the melanized melanosomes is transferred into cortical and medulla keratinocytes. During the anagenic stage of the hair growth cycle, this activity is regulated by a number of enzymes, structural and regulatory proteins, transporters, receptors and their ligands (Slominski *et al.*, 2004).

2.1.2 Drug Incorporation Routes

There have been several studies on the paths for drug incorporation into hair: from the bloodstream, from sweat and other secretions, and from external contamination. Figure 2.3 shows the model of drug incorporation into hair.

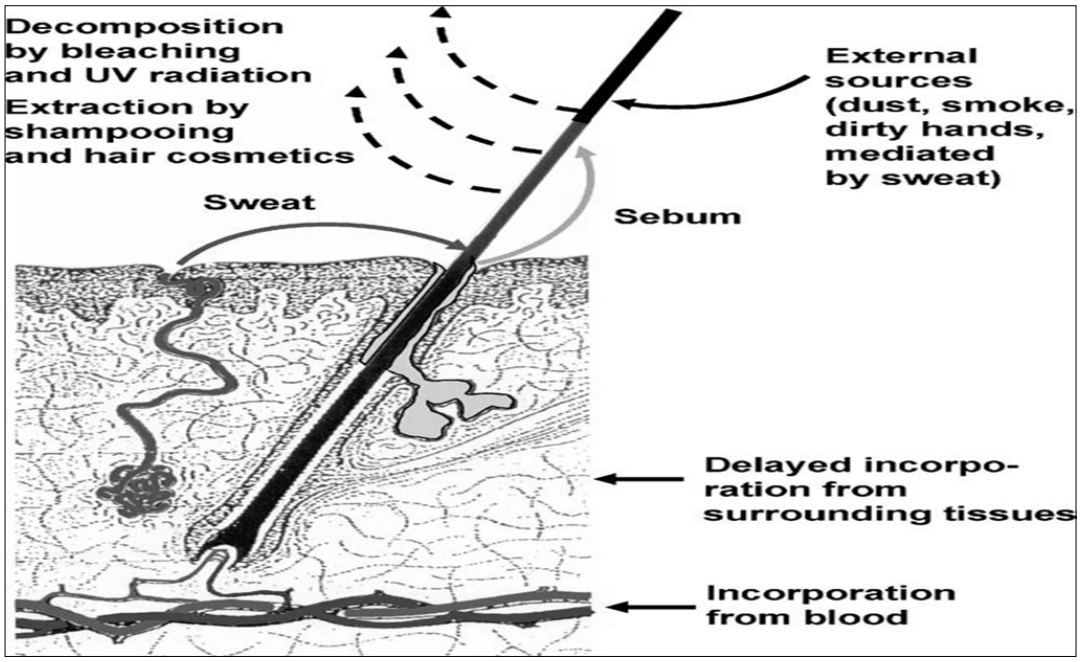

Figure 2.3 The models of drug incorporation in hair (Pragst & Balikova, 2006)

2.1.2.1 Incorporation from the Bloodstream

The passive transfer is the simplest model which describes drug incorporation into hair from the bloodstream (Boumba *et al.*, 2006; Pragst & Balikova, 2006). In this model, the hair follicle is provided with a sufficient blood supply, due to rapid cell division in the cells forming hair. Therefore, the drugs circulating in the blood will also reach the hair follicle. Firstly, a drug has to diffuse across the cell membrane to enter the matrix cells of the growing hair. The rate of this transport, where only drug molecules which are not bound to protein may be involved and this is further affected by the lipid solubility of the drug. In addition, the pH gradient between the plasma and the cell is critical for the transport (Kronstrand & Scott, 2006). Many drugs vary between either

weak bases or weak acids that can be protonated or deprotonated. The pH of plasma is 7.3, whereas the pH of the keratinocytes and melanocytes is lower, varying between 3 and 6 (Robbins, 2002).

2.1.2.2 Incorporation from Sweat and Other Secretions

There are several studies which support the evidence of incorporation of drugs from sweat and other secretion into hair. Amphetamine (Vree *et al.*, 1972), heroin, cocaine and metabolites (Cone *et al.*, 1994) and methadone (Henderson & Wilson, 1973) have been found in sweat, often in concentrations greater than blood. It is a fact that the drugs and metabolites are excreted in sweat. Hair is porous, and therefore absorbs liquid resulting in an increase in weight of up to 18% and due to this property; drugs may be transferred easily to hair through sweat. According to Henderson et al. (1996) in a study that examines the dose response relationships of deuterated cocaine into hair, it was found that deuterated cocaine was detected in multiple segments after single dose, upholding sweat or other secretions as a path for drug deposition in hair.

2.1.2.3 Incorporation from External Contamination

Environmental exposure (air, water, smoking or hair treatment) to drugs may contaminate hair specimens and drug users often defend positive hair testing results by claiming passive exposure to drugs. Before extraction of drugs, decontamination of hair specimens is very important because it removes any external contamination and is

essential to produce valid results. Joya, et al. (2009) reported that unsuspected exposure of cocaine in preschool children between 18 months and 5 years old using hair analysis and found 88% of positive cases for cocaine were also found in the hair of accompanying parents. This reflects the passive exposure to drugs. In another study, Garcia et al. (2009) observed that measurement of cocaine hair concentrations can allow estimation of the degree environmental drug exposure in young children. Koren et al. (1992) showed that pyrolysis of crack results in accumulation of cocaine in hair, but not its metabolite benzoylecgonine. In cocaine abusers, both cocaine and benzoylecgonine are detectable in hair. Washing may not remove all cocaine in hair accumulated from extensive environmental exposure. On the other hand, if intense washing procedures are used, they can lead to removal of drugs from hair, thus raising the chance of false negative results (Paulsen *et al.*, 2001; Romano *et al.*, 2001).

2.1.3 Factor Affecting the Content of Drugs on Hair

2.1.3.1 Effects of Hair Color

Melanin that determines hair colour is a polyanionic polymer of eumelanin and pheomelanin. Eumelanin concentration is highest in black colored hair whereas red hair has the highest concentration of pheomelanin. White hairs do not have melanin. It has been hypothesized that incorporation of various drugs into hair depends on the concentration of melanin. However, there are conflicting reports in the literature as to whether there is any significant difference between hair color as well as ethnicity and

incorporation of drugs into hair. Kronstrand et al. (2003) measured methamphetamine and amphetamine levels in patients receiving selegiline, the drug which metabolizes to methamphetamine and amphetamine. Methamphetamine and amphetamine in hair showed preference for pigmented hairs over white hair. Goldberger et al. (1998) found higher concentrations of cocaine in hair of people of African origin compared to the Caucasoid group. In contrast, Schaffer et al. (2005) studied cocaine incorporation in blonde, auburn, brown and black hair, and did not observe any difference between hair specimens of different colors. However, when hair was permed, there was an increase in cocaine uptake. A study on 1,852 applicants for employment that classified them as "black" or "white" showed no significant difference between hair and urine drug test results for these two classes (Hoffman, 1999).

2.1.3.2 Effects of Cosmetic Treatments

Significant concerns with regard to drug analysis in hair are the change in the concentration of drug-induced effects of the cosmetic treatment of hair. This treatment has strong chemical, physical and mechanical influence could have harmful effects on the cuticle: this includes perming, straightening, dyeing, bleaching, excessive washing, intensive illumination with ultraviolet radiation, excessive exposure to sunlight (Agius & Kintz, 2010; Panagotacos, 2005). Jurado et al. (1997) investigated the effects of cosmetic treatment (bleaching and dyeing) among cannabis and nicotine concentration in hair. Their findings support the hypothesis that the cosmetic treatment of hair

decreases concentrations of drug in all treated hair samples especially in cases of severely damaged hair.

2.1.4 Adulteration of Hair Specimens

Under direct supervision, the chances of adulteration during sample collection are low. The treatment of hair before sample collection can occur and may affect the results. There are a number of products available on the Internet which guarantees a negative drug test in hair. Rohrich et al. (2000) studied the effect of Ultra Clean on removing tetrahydrocannabinol (THC), codeine, morphine, cocaine, amphetamine, 3,4-ethylenedioxyamphetamine(MDA), heroin, 6-monoacetylmorphine (6-MAM), 3,4-methylenedioxymethamphetamine(MDMA), 3,4-methylenedioxyethylamphetamine (MDE), dihydrocodeine and methadone from postmortem hair samples with known histories of drug abuse. The authors found that none of the specimens tested negative after treatment with shampoo and there was only a slight decrease in drug concentrations compared to untreated specimens. The results showed that drug substances had not been sufficiently eliminated from human hair by single ultra clean treatment to cause negative results (Dasgupta, 2010a).

2.1.5 Collection Procedures

Collection procedures for hair analysis for drugs have not been properly arranged (Kintz, 2008). Among the widely published studies, the samples are acquired from random locations on the scalp, albeit, hair is picked suitably from the back of the head.

This is called the *vertex posterior* which is the best collection site. In comparison with other areas of the head, this area has lesser variation in the hair growth rate at approximately 85% (Kronstrand & Druid, 2006). The number of hair strands during the growing phase is more stable, and also less influenced by factors of age- and sex-related parameter. After collection, hair samples should be wrapped in aluminum foil to preserve the alignment, and root and tips ends must be distinguished. Depending on the drug to be analyzed and the test methodology, sample size varies significantly within laboratories and relies on the sample sizes highlighted in the literature; this range from a single hair to 200 mg (Kintz, 2008). When sectional analysis is conducted, the hair is cut into segments of about 1, 2, or 3 cm, which correlates roughly to about 1, 2, or 3 months' growth. When scalp hair is not available, other types of hair (pubic hair, arm hair, or axillary hair) can be collected as an optional source for drug detection.

2.1.6 Methodology of Hair Analysis

Hair analysis involves at least five steps: (a)decontamination of the hair,(b) preparation of hair: pulverization, segmentation in short pieces,(c) incubation in methanol, acid, sodium hydroxide, buffer,(d) extraction : liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase microextraction (SPME), and (e) analysis: immunoassay, screening or/and chromatography coupled to mass spectrometry (Boumba *et al.*, 2006; Pragst & Balikova, 2006; Srogi, 2006).

2.1.7 Applications of Hair Analysis

The applications of hair analysis are widely used in clinical applications, criminal investigations (drug-related deaths, drug-facilitated crime, child protection, etc.), for monitoring drug misuse such as drug rehabilitation programs, workplace, drug testing and driving license re-granting. The clinical applications of hair analysis include psychiatric patients, epileptic management and therapeutic drug monitoring (Cooper *et al.*, 2011; Pragst & Balikova, 2006).

2.2 Drugs of Abuse

Drug abuse remains a significant public health issue worldwide. It refers to the use of harmful or dangerous psychotropic substances, including alcohol and illicit drugs. The use of psychotropic substances leads to a dependency syndrome. This syndrome is defined as a group of behavioral and cognitive physiological phenomena, which arise after the repeated use of the material. Using such drugs leads to have a strong desire to keep taking them, have difficulties in controlling their use despite their harmful effects. Patterns of drug abuse in all parts of the world are constantly changing. They differ significantly on the basis of the region, from country to country, and to the geographical area within the same country. For example, widespread methamphetamine abuse is causing concern in the United States, Australia and Southeast Asia, but has less influence in northern Europe (Wills, 2005).

2.2.1 Amphetamine Type Stimulants

After cannabinoids **(Marijuana)**, amphetamines and designer drugs (Ecstasy) are the most consumed drugs. So, there has been great concern about the use of amphetamine type stimulants (ATS) as a public health issue. In recent years, the consumption of these drugs increased rapidly among the youth population as recreational drugs (Cirimele, 2006). Abuse of the powerful psychostimulants, amphetamine and methamphetamine is widespread in some geographical regions such as United States, Far East, Japan, with increasing consumption of designer drugs amphetamines being observed in European countries (Cirimele, 2006).

Amphetamines are Central Nervous Stimulant (CNS) and powerful synthetic psychostimulant drugs which increase the levels of norepinephrine, serotonin, and dopamine in the brain inducing emotion. Amphetamine-type stimulants (ATS) are collectively referred to several stimulants and hallucinogens chemically related to phenylethylamine (Dasgupta, 2010b).

Amphetamines are sympathomimetic amines that are often optically active. Amphetamine and any related designer are weak bases with low molecular weight that allows them to diffuse across membranes before being incorporated with nonconventional biological matrices, such as hair or nail. It has been noticed that using blood or urine for the purpose of measuring the level of drugs helps reflect a period of exposure that is over several hours or days before sampling. On the other hand, using

hair for drug detection allows the past medication history and drug abuse of an individual (Cirimele, 2006).

Amphetamine type stimulants (ATS) include amphetamine itself, dexamphetamine, methamphetamine, and fenfluramine. In addition to the amphetamines, there are other related compounds, such as methylphenidate, methylenedioxymethamphetamine (MDMA) and methylenedioxyamphetamine (MDA, or love pills). The focus of the present research is on a particular amphetamine, methamphetamine, MDA and MDMA. Figure 2.4 below illustrates the chemical structures of ATS.

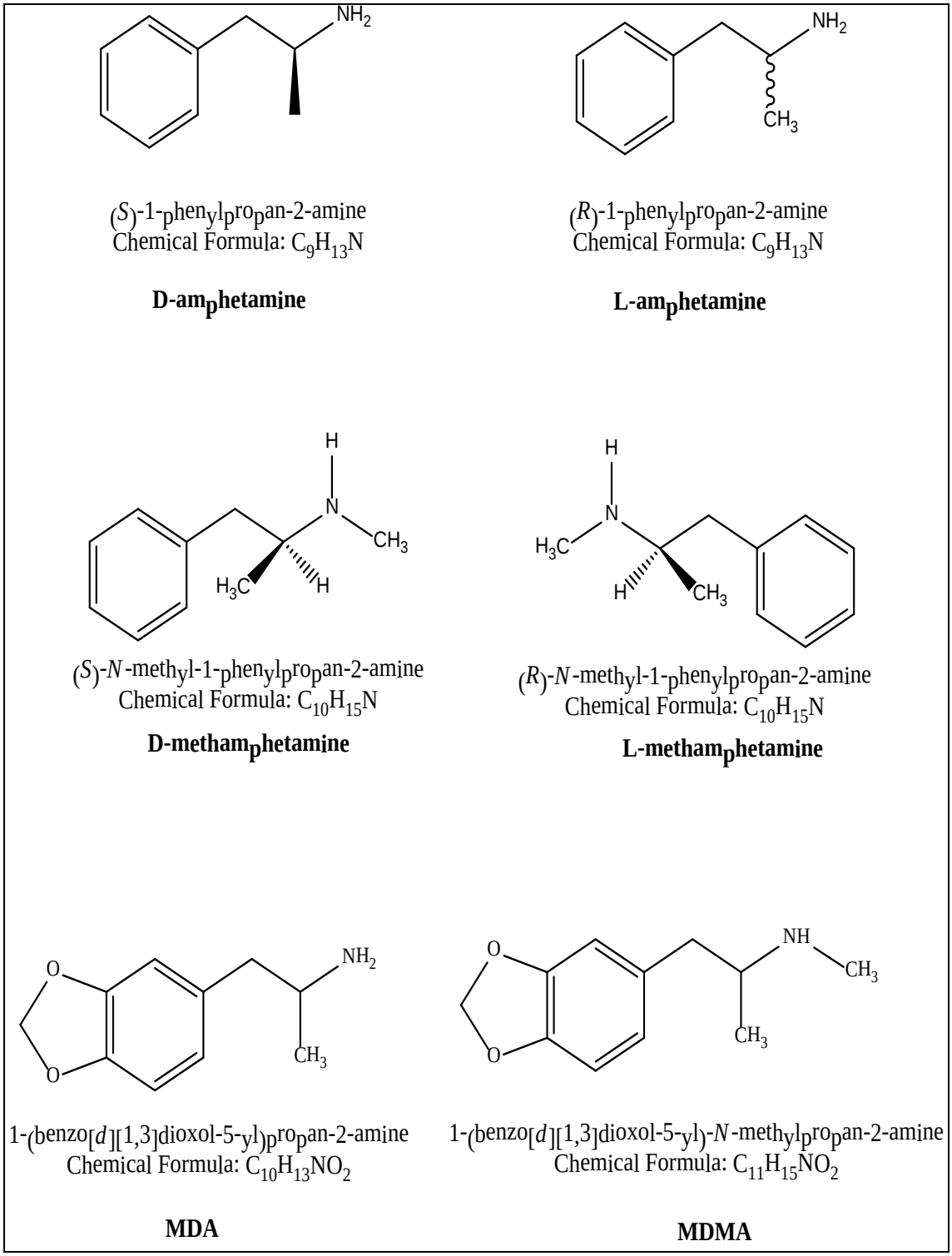

Figure 2.4 Chemical structures of amphetamine type stimulants (Freye & Levy, 2009; Wada *et al.*, 2010)

2.2.1.1 Physiochemical Properties

Both amphetamine and methamphetamine are chiral compounds. The racemic mixture can be divided into their optical isomers: levo (l) and dextro (d) isomeric forms. The d-amphetamine form has significant stimulant activity, and possesses approximately 3 to 4 times the central activity of the l-form. It is also important to note that the d- and l-enantiomers may not only have different pharmacological activity but also varying pharmacokinetic characteristics (Wills, 2005). The d-methamphetamine has significant central stimulant activity than the l-form and is commonly abused. However, l-methamphetamine has significant peripheral sympathomimethic activity and is typically found in non-prescription inhalers such as a decongestant. Because the free amphetamine and methampetamine molecules are rather insoluble oily liquid, they are usually handled as salts, such as methamphetamine hydrochloride. The salts are dissociated in the body, liberating the free amine to cause the known sympathomimetic effects (Freye & Levy, 2009).

3, 4-methylenedioxymethamphetamine (MDMA, ecstasy) is a ring substituted derivative of methamphetamine and one of the most abused designer drugs. This drug was synthesized by a chemist at Merck in 1914 as an appetite suppressant (Freye & Levy, 2009; Jenkins, 2006).

Another closely related designer drug, 3, 4-methylenedioxyamphetamine (MDA) is a potent psychotropic amphetamine derivative which was first synthesized in 1910. After 1986, a large number of amphetamine analogs were synthesized by clandestine laboratories to produce more potent effects after abuse (Jenkins, 2006). Table 2.1 shows the summary of physiochemical properties of Amphetamine Type stimulant (ATS).

2.2.1.2 Absorption

There is limited data is available on the gastrointestinal absorption of amphetamine in humans. Beckett and Rowland (1965) investigated serum concentrations of amphetamine in two healthy volunteers after a 15 mg oral dose of the d-isomer. He found that peak serum concentrations (48 and 40 ng/mL) were observed at 1.25h in acidic urine. Slightly higher serum concentrations were achieved (52 and 47 ng/mL) if the urine pH conditions were not controlled.

Absorption of methamphetamine by the oral route, dose of 5 to 10 mg typically result in blood concentrations of between 20 to 60 ng/mL (Jenkins, 2006). Peak methamphetamine concentrations occur at 3.6 h with a mean concentration of 20ng/mL after oral administered of a single dose of 0.125 mg/kg methamphetamine (Baselt & Cravey, 1995).